

# Possibilities of Thrombogenicity testing by *In Vitro* Systems

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# Topics to be addressed

- Pro's and Con's of in vitro models
- General overview of various available in vitro methods
- Flow loop model specifics
- In vitro assay validation
- Device geometry
- Investigate material changes in a marketed device

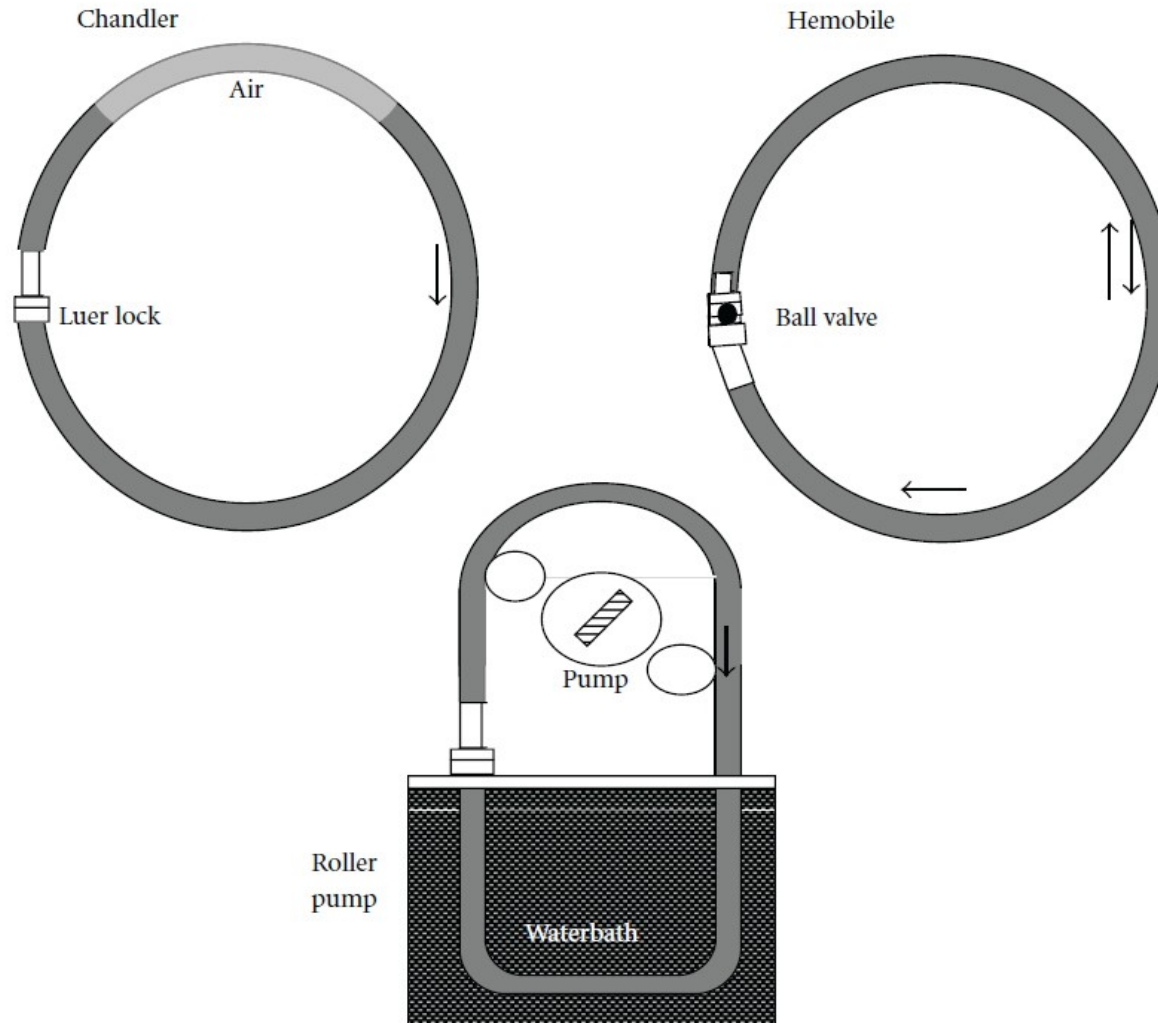
# Advantage small in vitro system

- Volume from 3,5 ml
- 1 Volunteer for all test and reference samples
- Reproducible per donor
- Puls, Flow and shear adjustable
- Low costs
- All immuno assays are commercially available (anti-human antibodies)
- Results of a complete study in short time
- Small materials for testing (from 0,09 cm<sup>2</sup>)
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# Limitations small in vitro system

- Long duration of testing not possible
- Effects endothelial cells ignored
- No feedback functions (from organs)
- No surgical effects (incl release factors)
- Anatomy differences
- Aspecific blood activation (drawing, circuit)
- Anticoagulant needed
- Effects exaggerated (ratio device/blood and accumulation)

# Blood circulation models



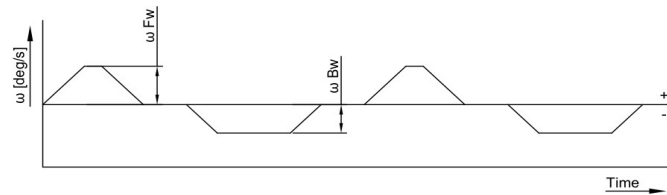
# Flow loop model specifics:

++ =good

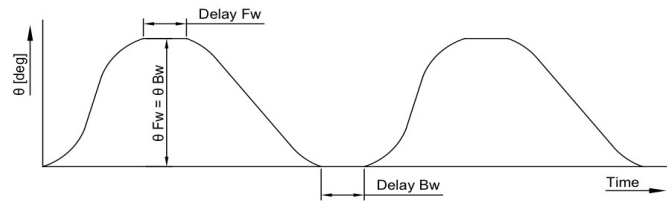
	Chandler	Pump	Hemobile
Making	++	++	+
Handling	++	—	++
Replicates	++	—	++
Flow/shear	—	++	+
Pulse	—	—	++
Intrinsic activation	++	—	++

# Hemobile specifics

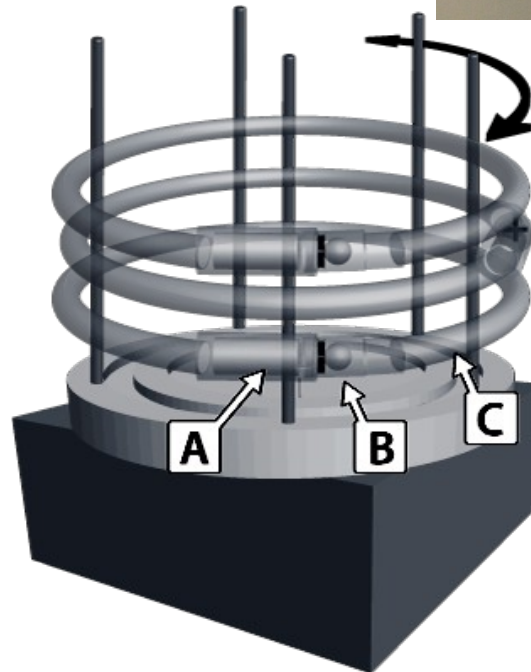
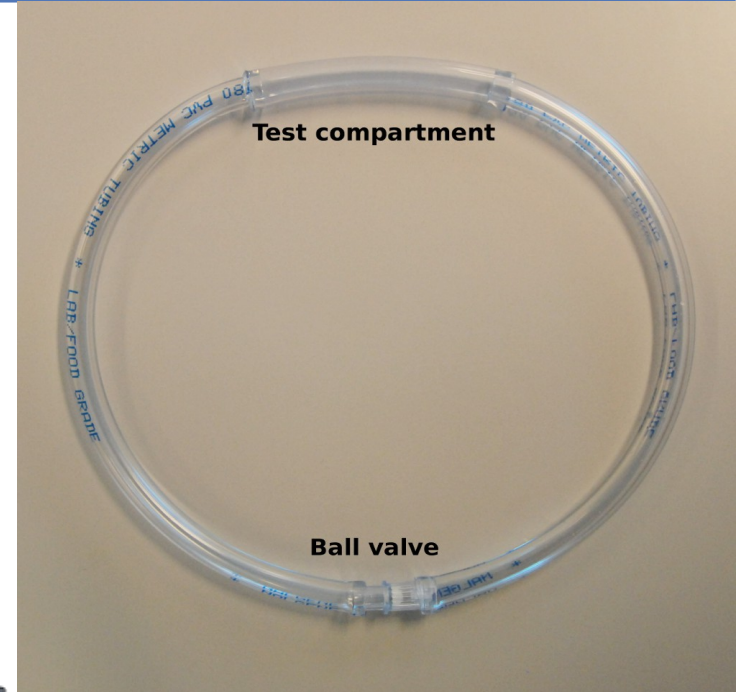
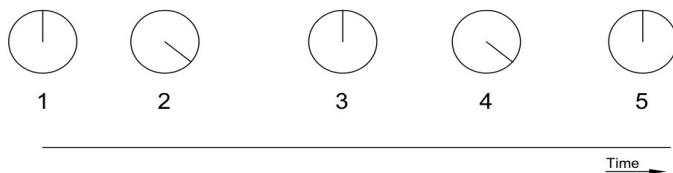
Angular velocity ( $\omega$ )



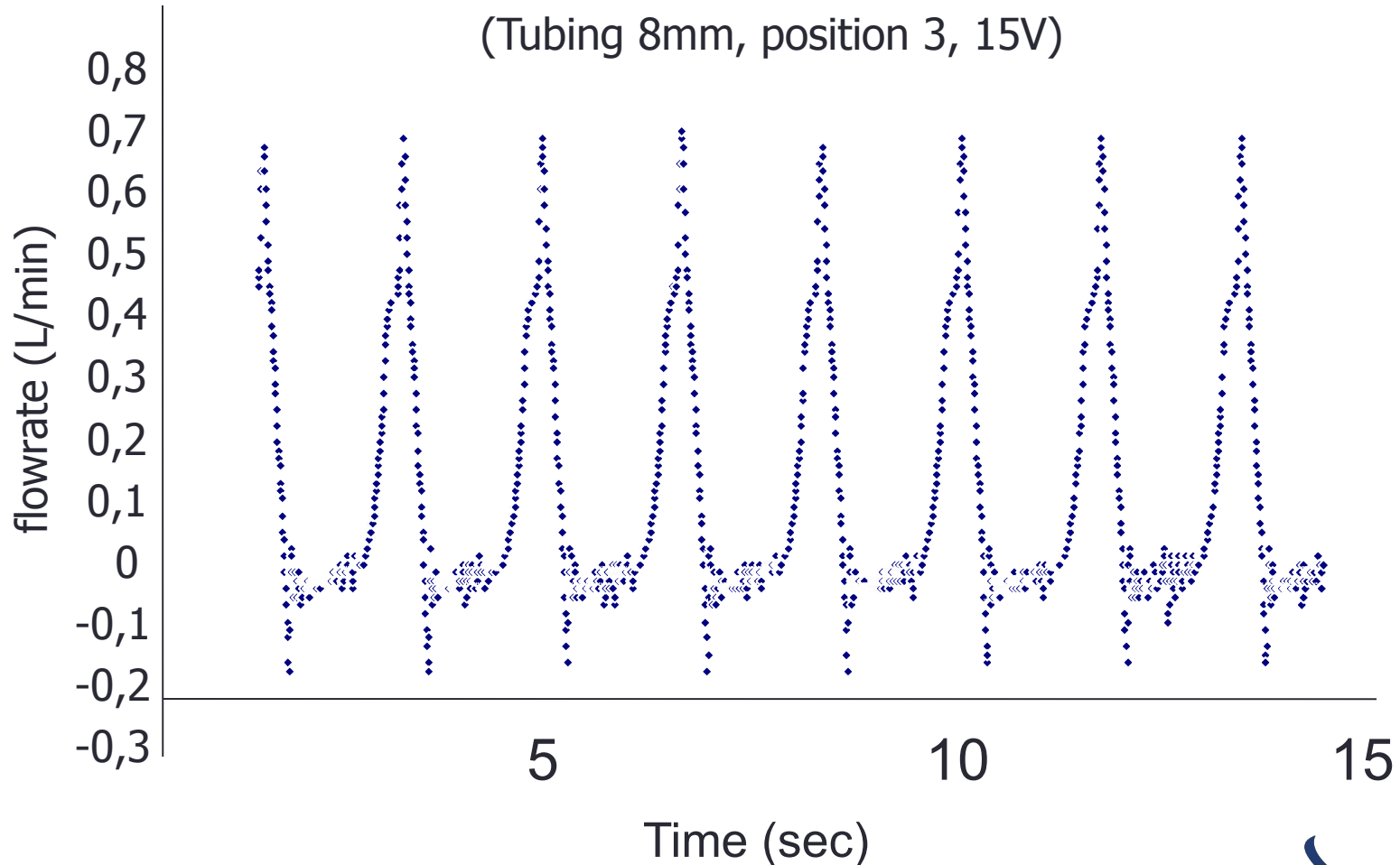
Angular displacement ( $\theta$ )



Haemobile position



## Haemobile adjusted to heart beat frequency, Doppler flow measurement on tubing

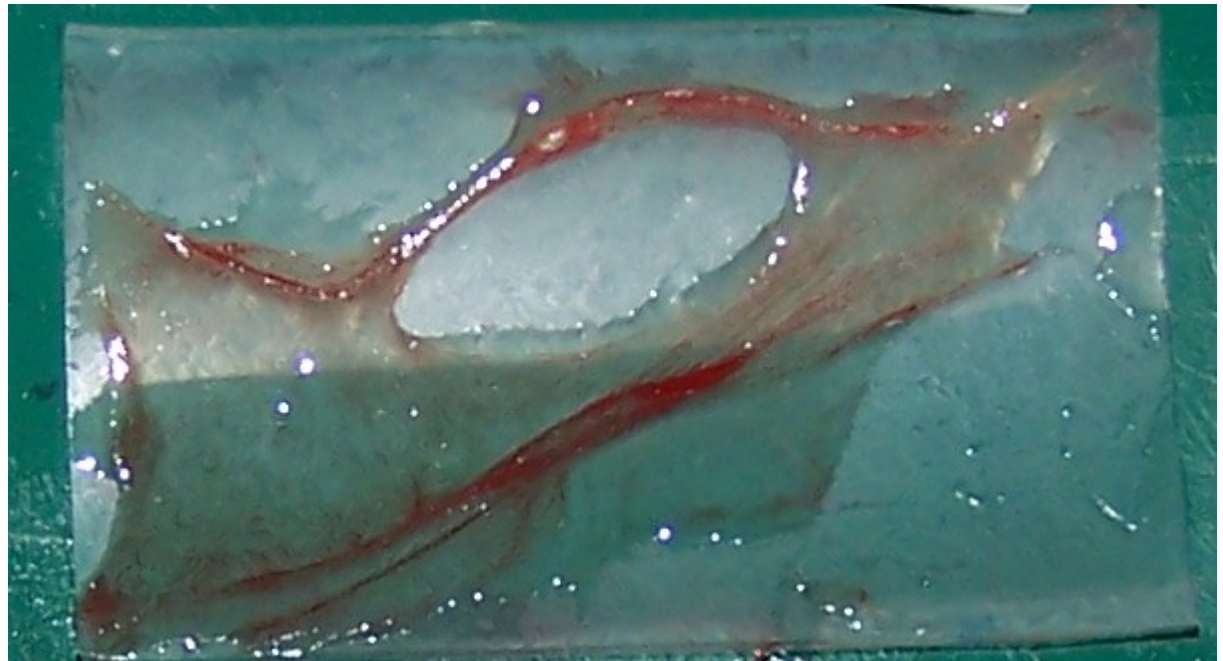




# Validation

- In vitro conditions (Repeatability, reproducibility, accuracy)

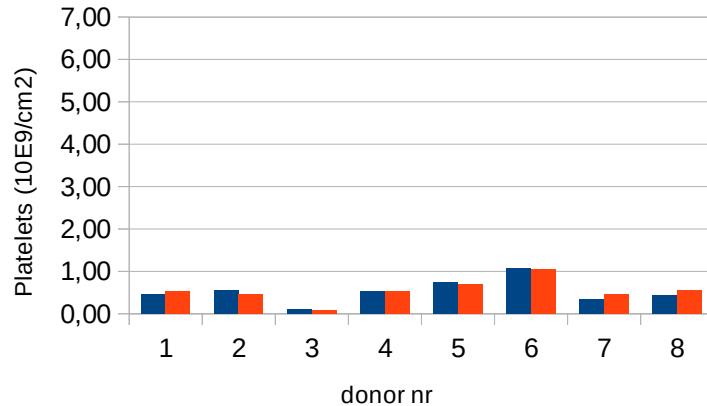
PDMS induced  
Thrombus formation:  
Detachment is possible



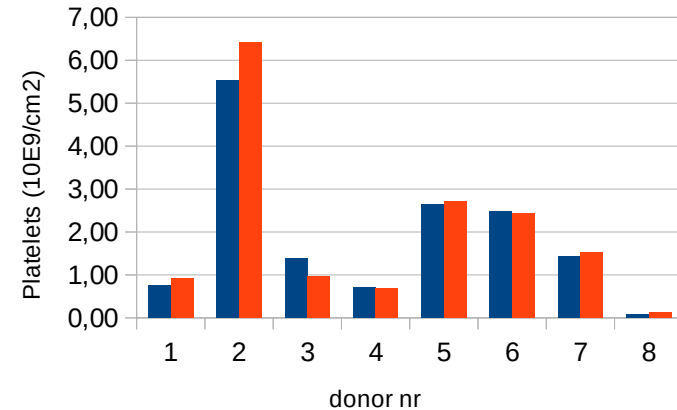
- Clinical effects

# Validation. In vitro conditions: reproducible

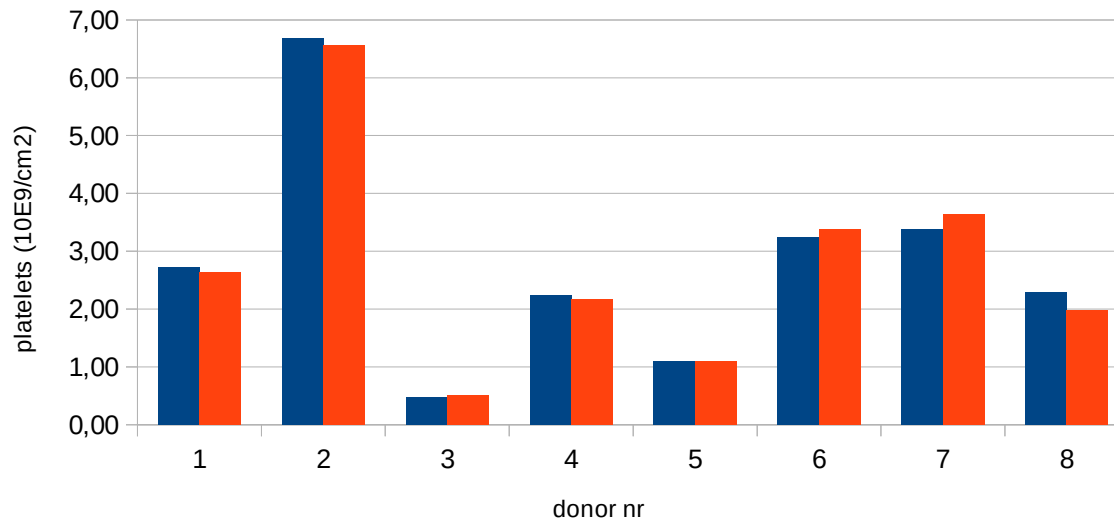
Platelet adhesion to PVC (duplicates)



Platelet adhesion to PDMS (duplicates)

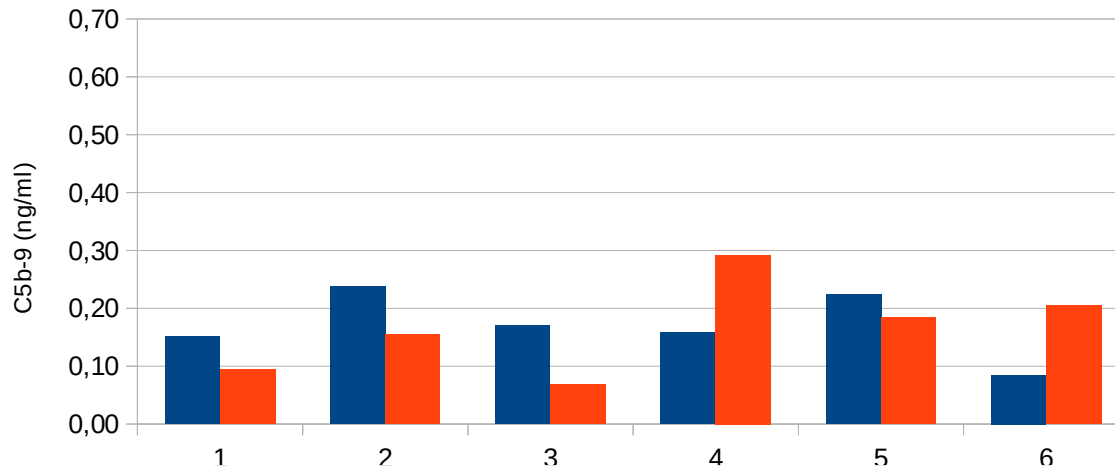


Platelet adhesion to PTFE (duplicates)

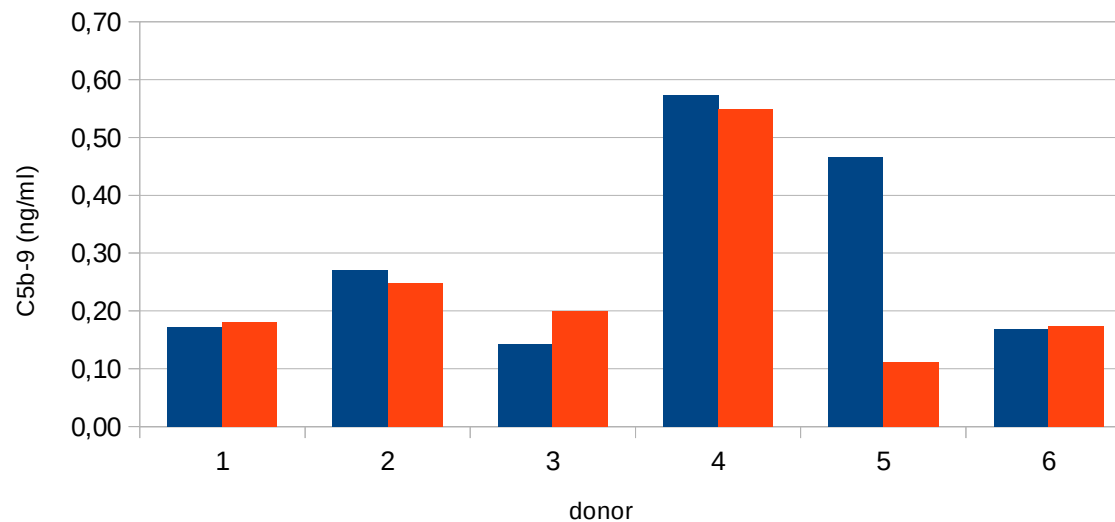


## Validation: reproducibility of complement and donor vari

Complement C5b-9 PVC



Complement C5b-9 PTFE



# Validation. Clinical effects

In vitro findings correspond with clinical observations

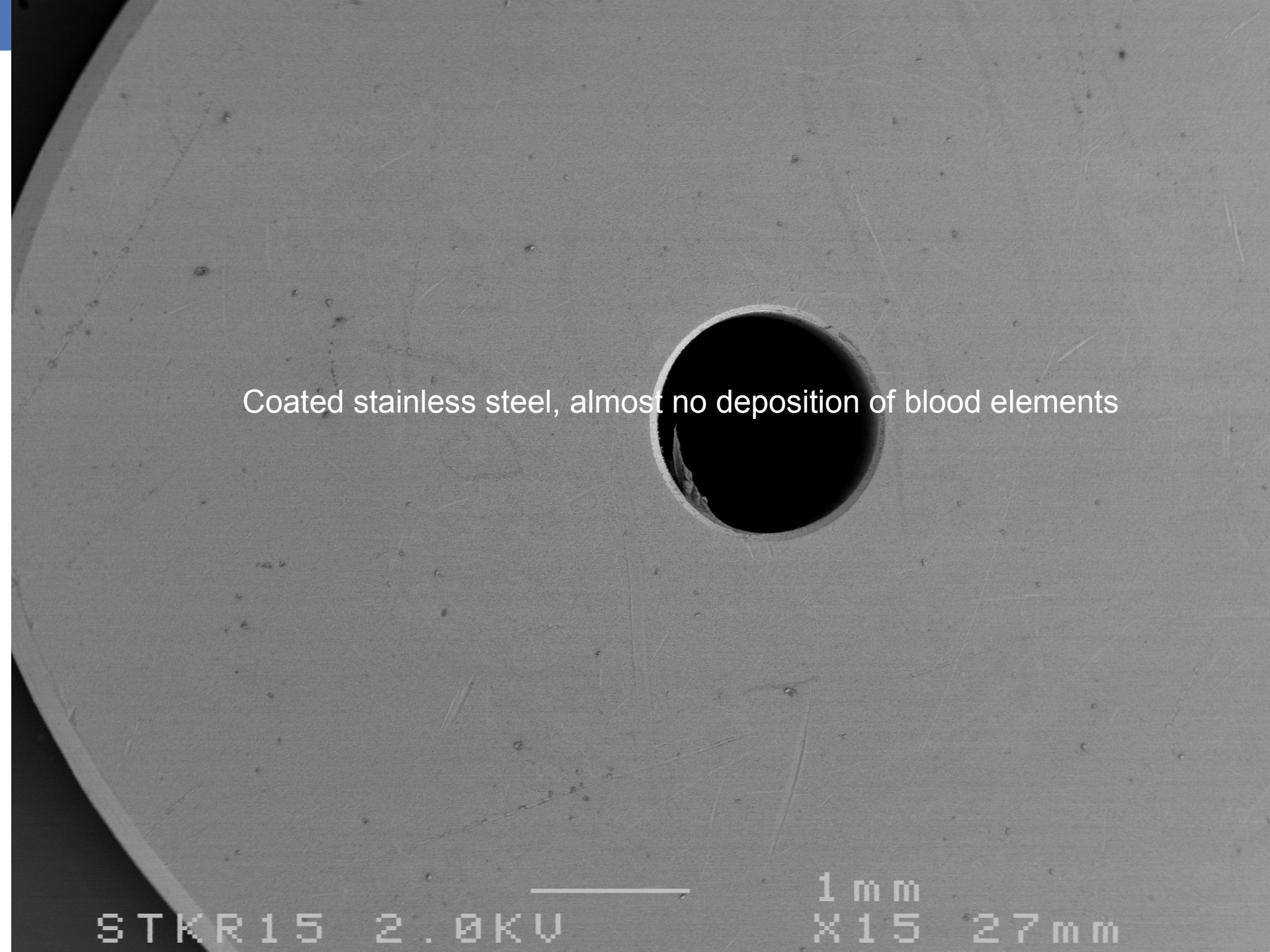
Examples:    Heparin coated stainless steel  
                 Carmeda coated extracorporeal circuit



Stainless steel without coating: platelet adhesion and fibrin

STK02 2.0KV 10µm X600 25mm





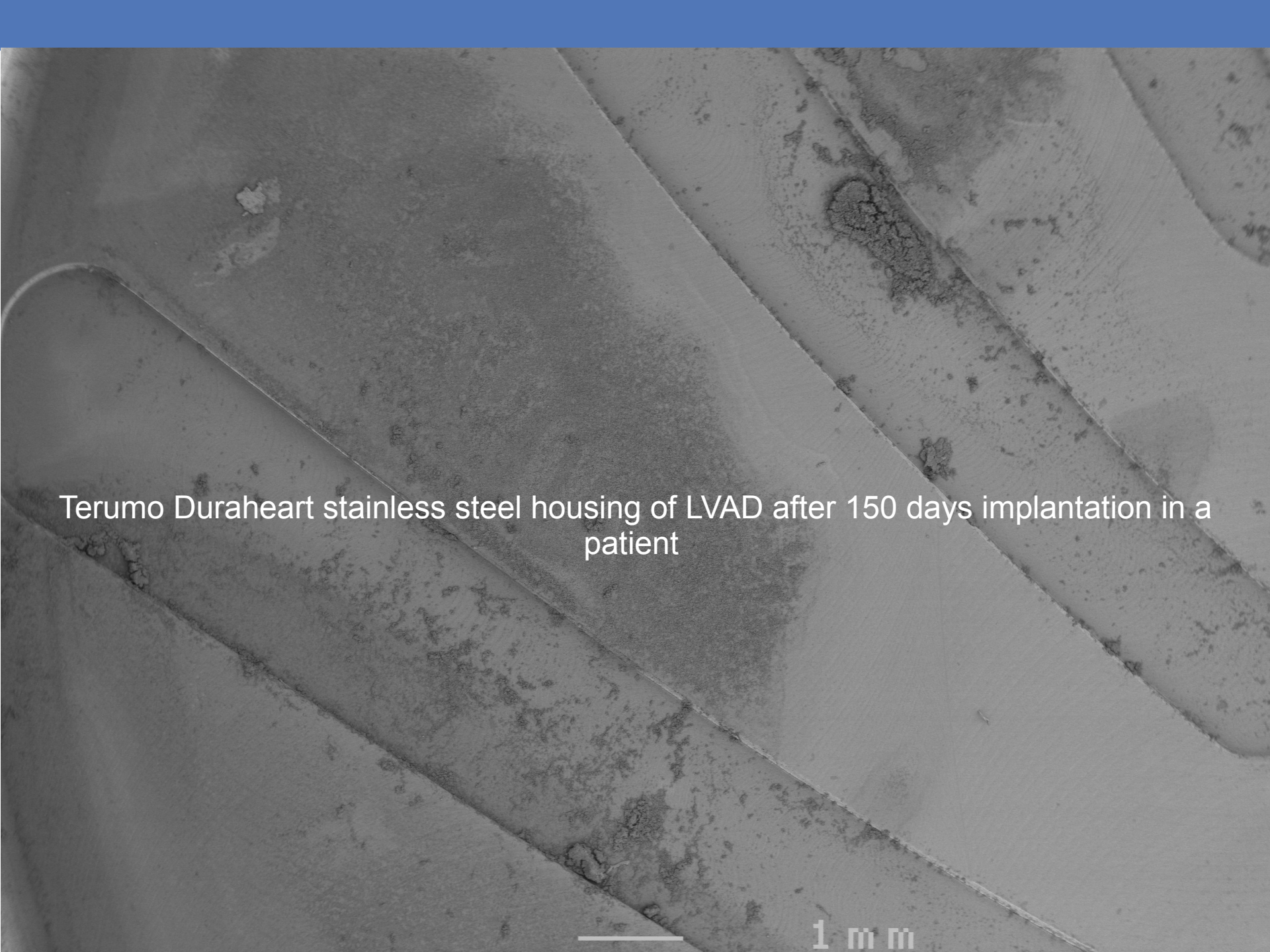
Coated stainless steel, almost no deposition of blood elements

This scanning electron micrograph (SEM) shows a circular, coated stainless steel disc. A central hole is visible, and the surface of the disc is covered with a fine, granular coating. The text overlay indicates that there is almost no deposition of blood elements on the surface. The image is in grayscale, highlighting the texture of the coating and the metallic surface of the disc.

STKR15 2.0KV

1mm  
X15 27mm





Terumo Duraheart stainless steel housing of LVAD after 150 days implantation in a patient

This scanning electron micrograph (SEM) shows the surface of a stainless steel LVAD housing. The surface is characterized by several diagonal, parallel ridges or grooves. There is significant surface degradation, including numerous small pits, larger irregular corrosion products, and areas of material loss, particularly along the edges of the ridges. The overall texture is rough and uneven.

1 mm



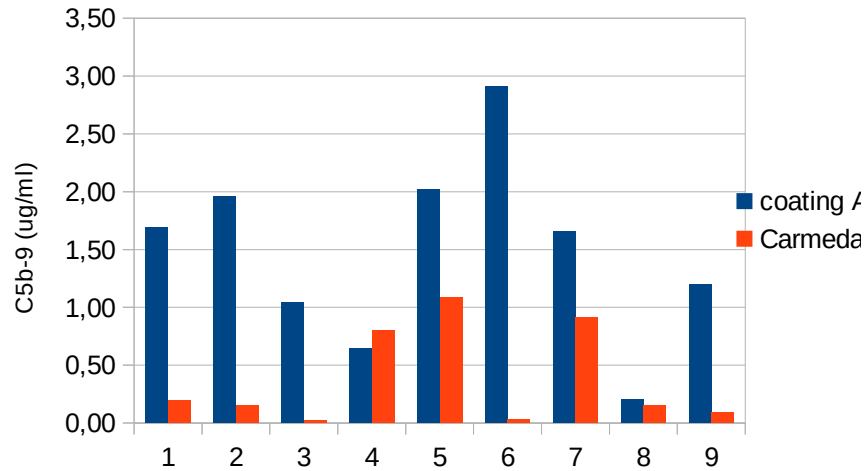
Uncoated stainless steel stent after 1 hour blood contact in vitro:  
Thrombus formation



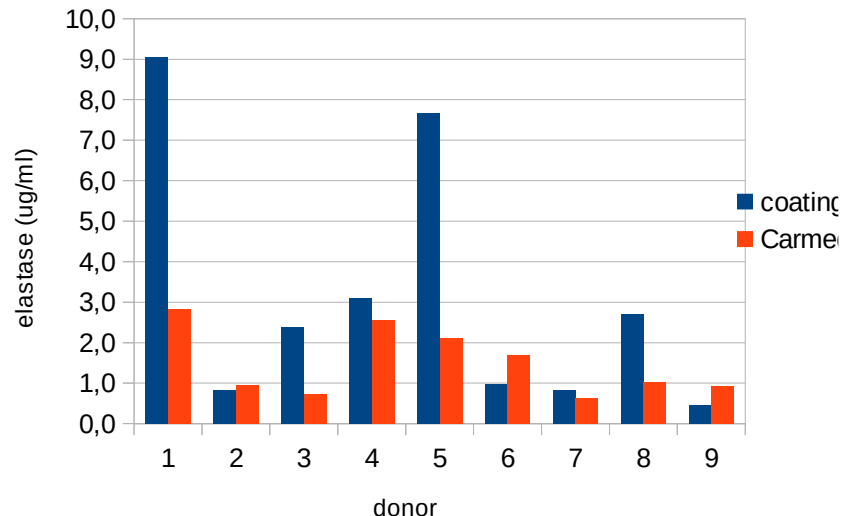


## Validation. In vitro results of Carmeda correspond to clinical observations: inhibition of complement activation and leukocyte activation

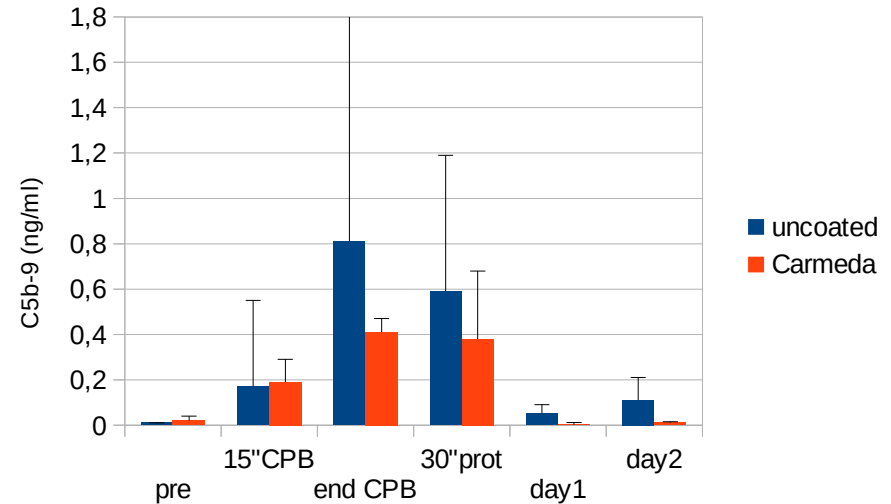
Complement activation in vitro (C5b-9)



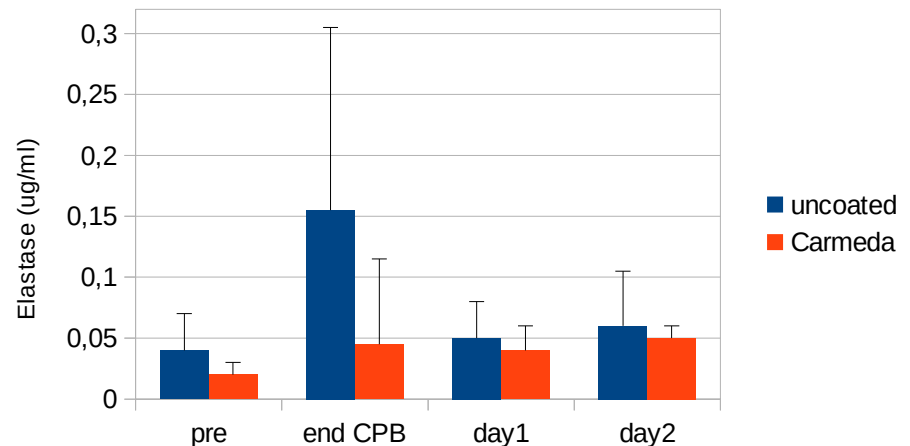
Elastase release in vitro



Complement activation in patients

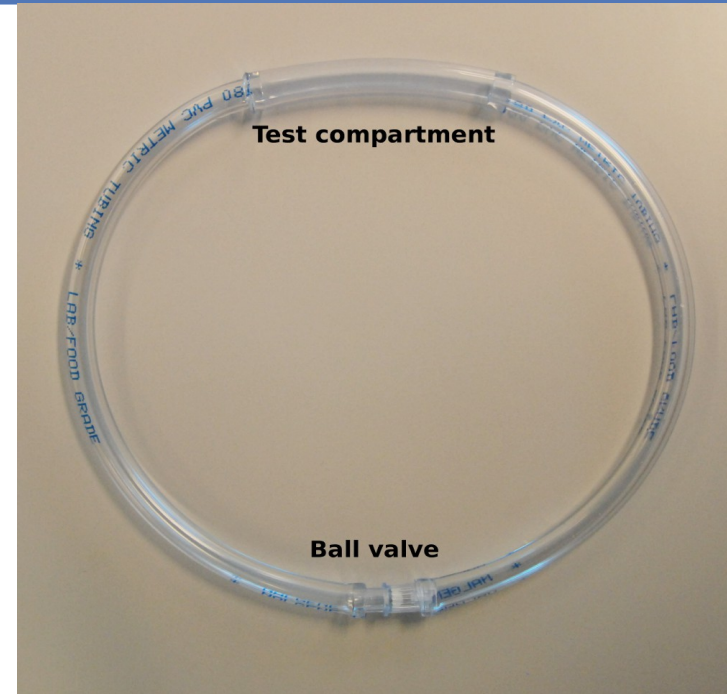


Elastase release in patients



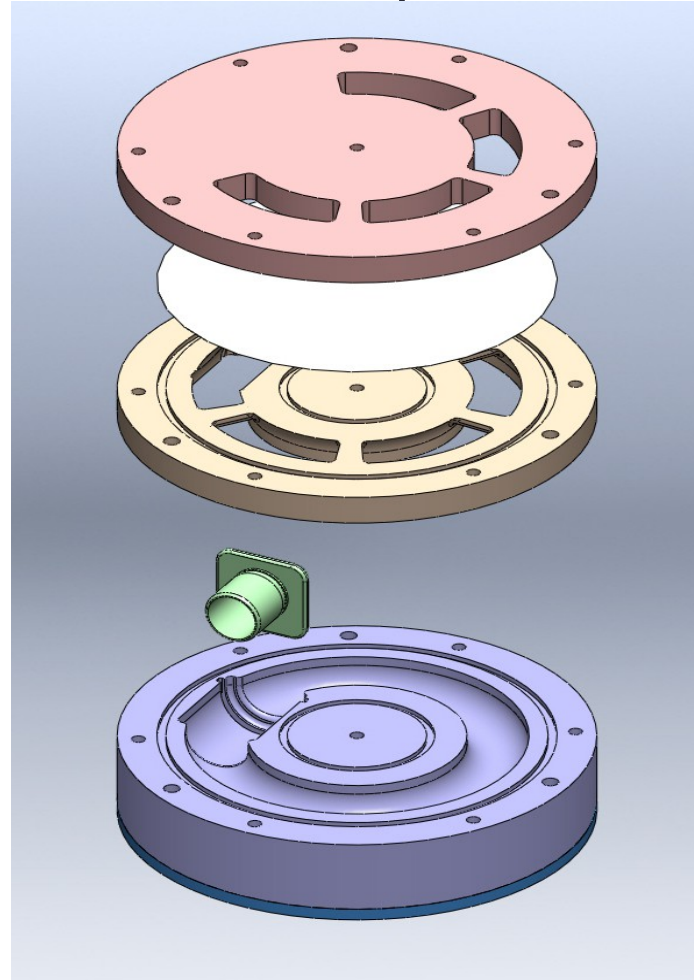
# Geometry

- Catheters, stents, vascular grafts in PVC tubing
- Heart valves: special chamber
- Other shapes: attached device

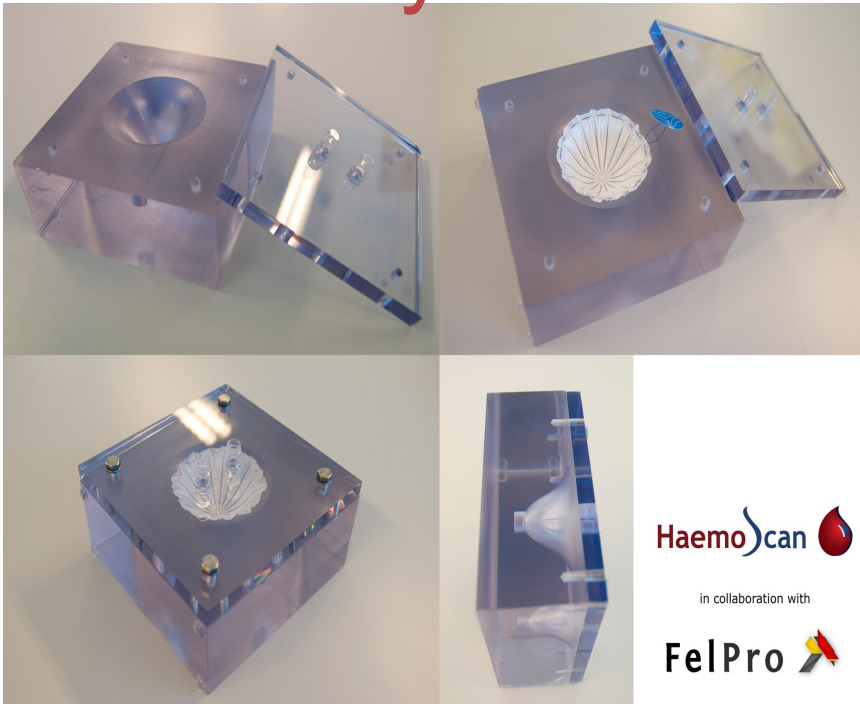


# Geometry

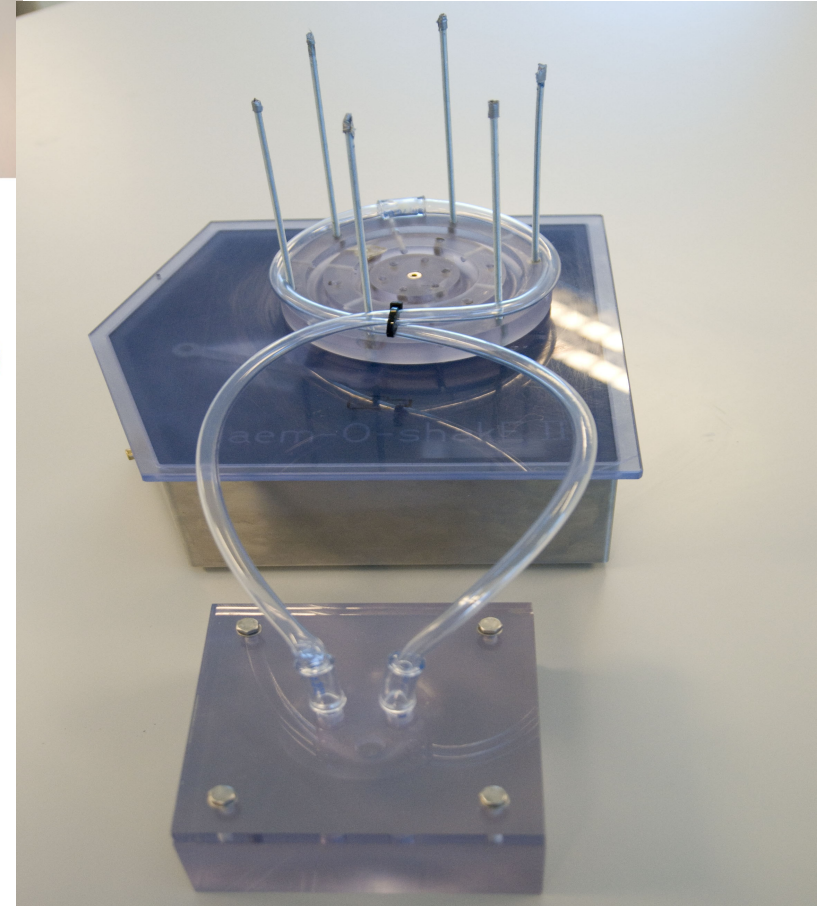
Heart valves can be mounted in a special device with a circular inner volume.



# Geometry



Testing of a left ventricle supporting device.  
Flow is generated with the Hemobile and is applied to the test chamber.



# Haemocompatibility testing ISO 10993-4

Five categories:

1. Thrombosis
2. Coagulation
3. Platelets
4. Hematology
5. Complement

## Choice of testing

	Thrombosis	Inflammation
Investigate material changes in a marketed device	SEM/Platelet adhesion/P-selectin count/aggregation/function Release products BTG, TxB2, serotonin	SEM/ Fibrin adhesion PTT/Thrombin generation TAT, FpA
		Convertase activity C5b-9, C3a, C5a Elastase CH50/AP50

# Preferred direct surface examination

**Thrombosis:** Scanning electron microscopy

**Coagulation:** Fibrin adhesion

**Platelets:** Platelet adhesion, P-selectin expression

**Inflammation:**

**Hematology:** Leukocyte binding (CD11)

**Complement:** C5-Convertase or C3b

Separate experiments (24 hrs): Hemolysis

# To be determined

Circulation time 4 hours, or 1 hour, or shorter time (platelet and complement react optimally within 30 minutes).



# Conclusion

In vitro systems are excellent tools to determine the material properties.

The small loop systems allow multiple testing incl references with blood from 1 (human) donor.

Human blood is different from animal blood and all assays can be done on human blood samples.

Differences between donors can be observed, which may lead to an estimate of number of donors needed.

Thrombogenicity testing by in vitro systems creates new possibilities